Exhibit B

UNITED STATES PATENT AND TRADEMARK OFFICE

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BEFORE THE PATENT TRIAL AND APPEAL BOARD

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ARIOSA DIAGNOSTICS

Petitioner,

v.

ISIS INNOVATION LIMITED

Patent Owner.

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IPR2013- _____

Patent 6,258,540

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PETITION FOR *INTER PARTES* REVIEW
UNDER 35 U.S.C. § 312 AND 37 C.F.R. § 42.104

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1001 (A)	U.S. Patent No. 6,258,540 to *Lo et al.*
1002 (B)	Intentionally left blank.
1003 (C)	Great Britain Patent Application No. 2,299,166
1004 (D)	Great Britain Patent Application No. 9,704,444
1005 (E)	First Declaration of David L. Barker
1006 (F)	First Declaration of Vasily Kazakov
1007 (G)	First Declaration of Elaine S. Mansfield
1008 (H)	First Declaration of Valeri Vasioukhin
1009 (I)	*Anker et al.*, *Spontaneous extracellular synthesis of DNA released by human blood lymphocytes*, Cancer Res 35:2375-2382 (1975).
1010 (J)	*Anker, et al.*, *Prevention and Detection of Cancer*, ed. Nieburgs, Marcel Dekker, Inc.1: 1-16 (1977).
1011 (K)	*Bianchi*, *Prenatal Diagnosis by Analysis of Fetal Cells in Maternal Blood*, J. Pediatr. 127:847-856 (1995).
1012 (L)	The Genetic Science Learning Center of the University of Utah states that "90% of Trisomy 21 cases, the additional chromosome comes from the mother's egg rather than the father's sperm." http://learn.genetics.utah.edu/content/disorders/whataregd/down (last accessed September 16, 2012).
1013 (M)	*Ho*, *HN et al.*, *Activation status of T and NK cells in the endometrium throughout menstrual cycle and normal and abnormal early pregnancy*; Hum Immunol. 49(2):130-6 (1996).
1014 (N)	*Kazakov*, *et al.*, *Extracellular DNA in the Blood of Pregnant Women*, Cytology (Tsitologia), 37(3): 232-236 (1995).
1015 (O)	*Leon et al.*, *Free DNA in the Serum of Cancer Patients and the Effect of Therapy*, Cancer Research 37:646-650 (1977).
1016 (P)	*Lo et al.*, *Presence of fetal DNA in maternal plasma and serum*, Lancet 350:485-87 (1997).

1017 (Q)	*Lo*, *et al.*, Clin. Chem. Lab. Med. 50(5):1-4 (2011).
1018 (R)	*Mansfield*, *E. S.*, *Diagnosis of Down syndrome and other aneuploidies using quantitative polymerase chain reaction and small tandem repeat polymorphisms*, Human Molecular Genetics 2(1): 43-50 (1993).
1019 (S)	*Narwoz*, *et al*., Nature Medicine 2:1035–37(1996)
1020 (T)	*Robbins*, *Pathological Basis of Disease*, 5th ed., Schoen, W.B. Saunders Company, Ch. 10, pp. 440-441 (1994). (Fetal Development).
1021 (U)	*Robbins*, *Pathological Basis of Disease*, 5th ed., Schoen, W.B. Saunders Company, Ch. 23, pp.1072-1088 (1994) (Gestational trophoblastic diseases).
1022 (V)	*Schallhammer*, *L et al.*, *Phenotypic comparison of natural killer cells from peripheral blood and from early pregnancy deciduas*. Early Pregnancy 3(1):15-22 (1997).
1023 (W)	*Shapiro et al.*, *Determination of circulating DNA levels in patients with benign or malignant gastrointestinal disease*, Cancer 51, 2116-2120 (1983).
1024 (X-0)	*Simpson*, *JL* and *Elias*, *S.*, *Isolation fetal cells from maternal blood*, JAMA 270 (19): 2357-2361 (1993).
1025 (X)	*Simpson*, *JL* and *Elias*, *S*, *Isolating fetal cells in maternal circulation for prenatal diagnosis*, Prenat. Diagn. 14(13):1229-42 (1994).
1026 (Y)	*Sorenson et al.*, *Soluble normal and mutated DNA sequences from single-copy genes in human blood*, Cancer Epidemiol. Biomarkers Prev3:67-71 (1994).
1027 (Z)	*Stroun et al.*, *Isolation and characterization of DNA from the plasma of cancer patients*, Eur. J. Cancer Clin. Oncol., 23:707-712 (1987).
1028 (AA)	*Stroun*, *et al.*, *International Review of Cytology*, Circulating Nucleic Acids in Higher Organisms 51:1-48 (1977).
1029 (AB)	Intentionally left blank.
1030 (AC)	*Vasioukhin*, *et al.*, *K-ras Point Mutations in the Blood Plasma of Patients with Colorectal Tumors*, Biotechnology Today, Challenges of Modern Medicine, (5:141-150 (1996).

1031 (AD)	Notice of Motion and Motion for Preliminary Injunction Case No. 3:11-cv-06391-SI, Aria Diagnostics v. Sequenom, Document 34, Filed 03/08/12.
1032 (AE)	Intentionally left blank.
1033 (AF)	Declaration of *Dr. Mark I. Evans* in Support of Motion for Preliminary Injunction, Aria Diagnostics v. Sequenom, Case 3:12-cv-00189-BEN-BGS Document 12-2 Filed 02/22/12.
1034 (AG)	Preliminary Injunction Order, Aria Diagnostics v. Sequenom, Case 3:11-cv-06391, Document 121.
1035 (AH)	Intentionally left blank.
1036 (AI)	Intentionally left blank.
1037 (AJ)	Intentionally left blank.
1038 (AK)	Intentionally left blank.
1039 (AL)	*Ashoor, L., et al., Fetal Fraction in Maternal Plasma Cell-Free DNA at 11–13 Weeks' Gestation: Effect of Maternal and Fetal Factors. Fetal Diagnosis and Therapy*, published online May 4, 2012, p. 1-7.
1040 (AM)	Nonconfidential Brief of Appellant Sequenom, Inc. in Federal Circuit Appeal No. 12-1531.
1041 (AN)	Petition for Inter Partes Review, dated September 17, 2012, requesting review of claims 1-2, 4, 5, 8, 19-22 and 24-25 of U.S. Patent No. 6,258,540, now styled, *Ariosa Diagnostics v. Isis Innovation Limited*, Case No. IPR2012-00022 (MPT).
1042 (AO)	Decision on Institution of *Inter Partes* Review, IPR2012-00022, Paper 24, dated March 19, 2013 .
1043 (AP)	*Bianchi et al., Fetal Cells in Maternal Blood: Determination of Purity and Yield by Quantitative Polymerase Chain Reaction*, American Journal of Obstetrics and Gynecology, 171(4):922-6 (1994).
1044 (AQ)	*Lo et al., Detection of single copy fetal DNA sequence from maternal blood*, Lancet; 335:1463-64 (1990).
1045 (AR)	*Lo et al., Prenatal sex determination by DNA amplification from maternal blood*, Lancet, ii: 1363-65 (1989).
1046 (AT)	Second Declaration of *Vasily Kazakov*

1047 (AU)	Second Declaration of *Elaine S. Mansfield*
1048 (AS)	*Mutter et al.*, *Molecular diagnosis of sex chromosome aneuploidy using quantitative PCR*, Nucleic Acids Research, 19:4203-4207 (1991).

I. INTRODUCTION

On September 28, 2012, Ariosa filed a Petition for *Inter Partes* Review requesting review of claims 1-2, 4, 5, 8, 19-22 and 24-25 of the '540 Patent, now styled *Ariosa Diagnostics v. Isis Innovation Limited*, Case No. IPR2012-00022 (MPT). In the Board's Decision on Institution of *Inter Partes* Review it was ordered that the September 28, 2012 petition was granted with respect to various challenges to claims 1-2, 4, 5, 8, 19-22 and 24-25.

This petition challenges additional claims which depend directly or indirectly from claim 1, namely, claims 3, 12, 13, 15 and 18. These claims recite the use of fetal specific primers (claim 3), the detection of the sex of the fetus (claim 12), determination of the concentration of the fetal nucleic acid (claim 13), including where the concentration is abnormal (claim 15), and detection of an aneuploidy based on the concentration (claim 18).

Claims 3, 12, 13, 15 and 18 are anticipated by *Lo* (1997), which according to the Decision has a reasonable likelihood of anticipating independent claim 1. Alternatively, these claims are rendered obvious by *Lo* (1997) when viewed in light of *Lo et al., Detection of single copy fetal DNA sequence from maternal blood*, Lancet; 335:1463-64 (1990)(Ex. 1044 - AQ); and *Lo et al., Prenatal sex determination by DNA amplification from maternal blood,* Lancet, ii: 1363-65 (1989)(Ex. 1045 - AR).

Claims 3, 12, 13, 15 and 18 are also rendered obvious by *Bianchi et al., Fetal Cells in Maternal Blood: Determination of Purity and Yield by Quantitative Polymerase Chain Reaction*, American Journal of Obstetrics and Gynecology, 171(4):922-6 (1994) (Ex. 1043 - AP) when viewed in combination with any of the references found by the Board to establish a *prima facie* case of unpatentability of claim 1 (*i.e.*, (i) *Kazakov*, (ii) *Lo* (1997) and (iii) *Simpson* taken in combination with *Schallhamer* and *Kazakov*).

Claims 3, 12, 13, 15 and 18 are further rendered obvious by *Mutter et al., Molecular diagnosis of sex chromosome aneuploidy using quantitative PCR, Nucleic Acids Research, 19:4203-4207 (1991) (Ex. 1048 - AS)* when viewed in combination with any of the references found by the Board to establish a *prima facie* case of unpatentability of claim 1.

Petitioner below provides detailed comparison of the claimed subject matter and the prior art. Claims 3, 12, 13, 15, and 18 are unpatentable in view of the prior art presented herein.

This petition separately and additionally addresses claim 8 of the '540 patent, which was also addressed in the September 28, 2012 petition. The Board's decision on the earlier petition found that Petitioner "had not set forth a reasonable likelihood that claim 8 is anticipated by Kazakov" because "it is unclear what was amplified by the B1 and C2 primers described in the Kazakov reference."

(Decision at 26) Petitioner submits herewith new evidence that the experiments performed in the Kazakov reference utilizing the B1 and C2 primer would have resulted in the amplification and detection of paternally inherited fetal nucleic acids from multiple chromosomes in addition to the Y chromosome. This evidence establishes a reasonable likelihood that claim 8 is anticipated by Kazakov.

II. MANDATORY NOTICES

Pursuant to 37 C.F.R. § 42.8(a)(1), Petitioner provides the following mandatory disclosures.

A. Real Party-In-Interest

The real-party-in-interest is Ariosa Diagnostics, Inc. Ariosa Diagnostics is not barred by operation of estoppel to submit this petition for *inter partes* review.

B. Related Maters

The '540 Patent is asserted in co-pending litigation captioned as *Ariosa Diagnostics v. Sequenom et al.*, N.D.Cal, Case No. 3:11-cv-06391. The patent owner is currently appealing the district court's denial of its motion for a preliminary injunction.

On September 28, 2012, Ariosa filed a Petition for *Inter Partes* Review requesting review of claims 1-2, 4, 5, 8, 19-22 and 24-25. In the Board's Decision on Institution of *Inter Partes* Review, IPR2012-00022, Paper 24, dated March 19,

2013 ("Decision")(Ex. 1042 - AN), it was ordered that the Petition was granted with respect to various challenges to claims 1-2, 4, 5, 8, 19-22 and 24-25.

C. Lead and Back-Up Counsel

Pursuant to 37 C.F.R. § 42.8(b)(3), Petitioner provides the following designation of counsel: Lead counsel is Greg Gardella (Reg. No. 46,045). Scott A. McKeown (Reg. No. 42,866) and Kevin B. Laurence (Reg. No. 38,219) are back-up counsel.

D. Service Information

Pursuant to 37 C.F.R. § 42.8(b)(4), papers concerning this matter should be served on the following.

Address:	Greg Gardella Scott McKeown Kevin Laurence Oblon Spivak 1940 Duke Street Alexandria, VA 22314
Email:	cpdocketgardella@oblon.com cpdocketmckeown@oblon.com cpdocketlaurence@oblon.com
Telephone:	(703) 413-3000
Fax:	(703) 413-2220

III. PAYMENT OF FEES

The undersigned authorizes the Office to charge $23,000 to Deposit Account No. 15-0030 as the fee required by 37 C.F.R. § 42.15(a) for this Petition for i*nter partes* review. The undersigned further authorizes payment for any additional fees that might be due in connection with this Petition to be charged to the above referenced Deposit Account.

IV. REQUIREMENTS FOR *INTER PARTES* REVIEW

As set forth below and pursuant to 37 C.F.R. § 42.104, each requirement for *inter partes* review of the '540 Patent is satisfied.

A. Grounds for Standing

Ariosa certifies that this patent is available for *inter partes* review and that Ariosa is not barred or estopped from requesting an *inter partes* review challenging these claims on the grounds identified in this petition. Although Ariosa was served more than one year ago with a complaint asserting infringement of this patent, the normal statutory one-year bar under 35 U.S.C. § 315(b) does not apply here because (1) the Board has already instituted an *inter partes* review trial on this patent on a timely first petition filed by Ariosa (Case No. IPR2012-00022), and (2) Ariosa accompanies this second petition with a motion for joinder under 35 U.S.C. § 315(c).

B. Identification of Challenge

Pursuant to 37 C.F.R. §§ 42.104(b) and (b)(1), Petitioner requests *inter partes* review of claims 3, 8, 12, 13, 15 and 18 of the '540 Patent, and that the Patent Trial and Appeal Board ("PTAB") invalidate the same.

1. The Specific Art and Statutory Ground(s) on Which the Challenge Is Based

Pursuant to 37 C.F.R. § 42.104(b)(2), *inter partes* review of the '042 patent is requested in view of the following references, each of which is prior art to the '540 Patent under 35 U.S.C. § 102 (a), (b), and/or (e):

a. *Kazakov (Ex. 1014 - N)* and *Bianchi et al., Fetal Cells in Maternal Blood: Determination of Purity and Yield by Quantitative Polymerase Chain Reaction, American Journal of Obstetrics and Gynecology*, 171(4):922-6 (1994) (Ex. 1043 - AP), referred to herein as Bianchi, render obvious claims 3, 12, 13, 15 and 18.

b. *Kazakov (Ex. 1014 - N)* and *Mutter et al., Molecular diagnosis of sex chromosome aneuploidy using quantitative PCR, Nucleic Acids Research, 19:4203-4207 (1991) (Ex. 1048 - AS)*, referred to herein as *Mutter*, render obvious claims 3, 12, 13, 15 and 18.

c. *Lo (1997)(Ex. 1016 - P)* anticipates claims 3, 12, 13, 15 and 18.

d. *Lo (1997)(Ex. 1016 - P)* ; *Lo et al., Detection of single copy fetal DNA sequence from maternal blood*, Lancet; 335:1463-64 (1990)(Ex. 1044 -

AQ), referred to herein as *Lo (1990)*; and *Lo et al., Prenatal sex determination by DNA amplification from maternal blood*, Lancet, ii: 1363-65 (1989)(Ex. 1045 - AR), referred to herein as *Lo (1989)*, render obvious claims 3, 12, 13, 15 and 18.

e. *Simpson* (*Ex. 1025 -X*), *Schallhammer* (*Ex. 1022 - V*) and *Kazakov* (*Ex. 1014 - N*) and *Bianchi (Ex. 1043 - AP)* render obvious claims 3, 12, 13, 15 and 18.

f. *Simpson* (*Ex. 1025 -X*), *Schallhammer* (*Ex. 1022 - V*) and *Kazakov* (*Ex. 1014 - N*) and *Mutter (Ex. 1048 - AS)* render obvious claims 3, 12, 13, 15 and 18.

g. *Kazakov (Ex. 1014 - N)* anticipates claim 8.

2. How the Construed Claims Are Unpatentable under the Statutory Grounds Identified in 37 C.F.R. § 42.104(b)(2) and Supporting Evidence Relied upon to Support the Challenge

Pursuant to 37 C.F.R. § 42.104(b)(4), an explanation of how claims 3, 8, 12, 13, 15 and 18 of the '540 Patent are unpatentable under the statutory grounds identified above, including the identification of where each element of the claim is found in the prior art, is provided in Section VII, below, in the form of claim charts. Pursuant to 37 C.F.R. § 42.104(b)(5), the exhibit numbers of the supporting evidence relied upon to support the challenges and the relevance of the evidence to the challenges raised, including identifying specific portions of the evidence that

support the challenges, are provided in Section VII below, in the form of claim charts.

V. FACTUAL BACKGROUND

A. Declaration Evidence

This Petition is supported by the declarations of Professor Kazakov (*Ex. 1046 - AT*), Dr. Mansfield (*Ex. 1047 - AU*), Professor Kazakov (*Ex. 1006 - F*) and Dr. Mansfield (*Ex. 1007 - G*) from the September 28, 2012 petition. Unless specific reference is made to the declarations from that first Petition, this second Petition relies on the second declaration of Professor Kazakov (*Ex. 1046 - AT*) and the second declaration of Dr. Mansfield (*Ex. 1047 - AU*). Each offers various opinions with respect to the content and state of the prior art.

Professor Kazakov teaches the course Medical Genetics, Molecular Cloning and Genetic Engineering to the students pursuing Baccalaureate's and Master's degrees at the Department of Physico-Chemical Biology of Cells at the College of Medical Physics of the St. Petersburg State Polytechnical University. Professor Kazakov has been widely published in the field of cytology and molecular biology in refereed journals. Significantly, Professor Kazakov was the lead author of one of the primary prior art references relied upon in this Petition: *Kazakov et al.*, *Extracellular DNA in the Blood of Pregnant Women*, Cytology (Tsitologia)37(3): 232-236 (1995)(*Ex. 1014 - N*).

Dr. Elaine S. Mansfield is the author of another prior art publication, *Diagnosis of Down syndrome and other aneuploidies using quantitative polymerase chain reaction and small tandem repeat polymorphisms*, Human Molecular Genetics 2: 43-50 (1993)(*Ex. 1018 - R*). Dr. Mansfield has over thirty years of experience in genetic testing. Dr. Mansfield has worked both on the academic side, at Stanford University, and in the private sector, as an associate director of R&D at Affymetrix. Dr. Mansfield repeated certain experiments set forth in the *Kazakov* prior art reference and the details of those experiments are set forth in her declaration.

B. Most Cells in Circulation Release DNA into the Host Plasma

Most if not all cells in circulating in the blood necessarily release DNA into the bloodstream, and thus into the host plasma which is the cell-free portion of blood. Cells regularly release DNA through lysis, apoptosis (programmed cell death) and damage from collisions with other cells. (*Mansfield Decl. ¶ 86*) (*Ex. 1047 - AU*). After rupturing, the contents of the nucleus, including the DNA, are released into the host plasma. This DNA is also present in serum from the host, which is obtained by removing the clotting factors from the host plasma. (*Mansfield Decl. ¶ 85*) (*Ex. 1047 - AU*). The existence of such extracellular DNA released from circulating cells was widely reported in the context of various physiological states. (*Mansfield Decl. ¶ 85*) (*Ex. 1047 - AU*).

In the 1970s Anker *et al.*, *Spontaneous extracellular synthesis of DNA released by human blood lymphocytes*, Cancer Res 35:2375-2382 (1975) reported that "[h]uman blood lymphocytes released DNA *in vitro* in the absence of any stimulation." Anker further reported that extracellular DNA was released into bodily fluids in animal models. *(Anker, et al. (1977)(Ex. 1010 - J)*. Also in the 1970's several groups reported that increased levels of DNA were found in host plasma when the host had chronic diseases such as lupus, hepatitis and cancer. For instance, in 1977 Stroun *et al.* published a review entitled *Circulating Nucleic Acids in Higher Organisms*, in the International Review of Cytology, Volume 51 which offered the following commentary:

> At first sight it may have seemed premature to consider reviewing this topic, but it appears that sufficient evidence is available to demonstrate a physiologically controlled release of both DNA and RNA from living eukaryote cells, as well as from prokaryotes. This evidence has accrued from widely diverse cell types, such that it is likely to be a general event in eukaryotes, a point that needs to be further tested.

(Ex. 1028 - AA, p. 42, IV. General Comments, 1st ¶)

In the mid-1990s it was reported that pregnant females had elevated levels of extracellular DNA in their bloodstream. (*Kazakov et al.* (*1995*)(*Ex. 1014 - N*). Kazakov observed an increase in the concentration of extracellular DNA in the blood of pregnant women, with this increase being most strongly manifested in

association with gestosis (otherwise known as "preeclampsia"), a situation believed to involve the death of decidual cells by apoptosis. (*Id. at p. 234, 1st full* ¶). Kazakov observed this increase of extracellular DNA in both male and female fetuses (*Id. At Kazakov Decl. ¶35*) *(Ex. 1046 - AT)).*

As shown by the foregoing literature and as confirmed by the declarations of Professors Kazakov and Dr. Mansfield, at the time of the earliest claimed priority date (March 4, 1997) it was well known that nucleic acids were released into the plasma by cells that circulate in the bloodstream. This was the consistent and uniform observation for at least the majority of – and perhaps virtually all – cell types found circulating in the bloodstream.

C. It Was Well Known that Fetal Cells Were Found in the Maternal Circulation

In its background section, the '540 Patent (*Ex. 1001 - A*) admits that it was "well recognized" that fetal cells were found circulating in the mother's blood stream and that these cells were useful for prenatal testing:

> The passage of nucleated cells between the mother and foetus is now a well-recognized phenomenon (Lo et al. 1989; Lo et al. 1996). The use of foetal cells in maternal blood for non-invasive prenatal diagnosis (Simpson and Elias 1993) avoids the risks associated with conventional invasive techniques.

Because it was widely accepted that fetal cells were in circulation and could be isolated and genetically tested with PCR, in the early 1990s Dr. Mansfield

developed and published techniques for detecting genetic anomalies associated with fetal conditions. (*Mansfield* (*1993*)(*Ex. 1018 - R*). In her 1993 publication, Dr. Mansfield noted that publications as early as the 1980s suggested fetal cells were in maternal circulation and could be isolated and tested. (*Id. at p. 43*). Dr. Mansfield proposed quantitative PCR techniques that could be used to detect certain short tandem repeats associated with Down syndrome and Turner syndrome from these fetal cells in the maternal circulation. (*Id. at pp. 44-46*).

D. The Maternal Immune System Was Expected to Destroy Fetal Cells in Maternal Circulation

Prior to the priority date of the '540 Patent, uterine natural killer cells (uNK cells) were known to have significant differences from natural killer cell populations (NK cells) found in peripheral blood. (*Schallhammer* (*1997*)(*Ex. 1022 - V*). In particular, uNK cells produce very different proteins on their cell surface, and thus have different reactivity to other cells, including fetal cells. (*Mansfield Decl. at ¶ 85-96*) (*Ex. 1047 - AU*). In particular, the ability of uNK cells to kill other cells in the uterine lining is decreased in early normal pregnancies. (*Id. at ¶¶ 89-90*). This was believed to be at least in part responsible for the immune tolerance of the developing fetus displayed by the mother's immune system. (*Id. at ¶ 89*).

As explained in the Declaration of Dr. Mansfield, while cells at the

maternal-placental interface were known to be somehow protected by an immune suppressive mechanism, it would not have been expected that fetal cells in the circulating bloodstream would be afforded such protection. (*Id. at ¶ 90*). Thus it would have been expected that maternal NK cells in the mother's bloodstream would have recognized the fetal cells (*e.g.*, trophoblasts) as "non-self" and would destroy the circulating fetal cells and in the process release cell-free DNA into the plasma. (*Id. at ¶ 90*).

E. PCR Techniques Available in 1997 Could Detect the Presence of a Fetal Nucleic Acid Sequence

Dr. Lo's 1997 article is representative of a contemporaneous body of prior art which establishes that PCR could be used to determine the presence of a specific fetal sequence in a maternal sample. (*Lo* (1997)(*Ex. 1016 -P*). *Lo* (1997) discloses the use of fetal sequence-specific oligonucleotide primers as do several other references including *Bianchi* (*Ex. 1043 - AP*), *Mutter* (*Ex. 1048 - AS*), *Lo* (1990)(*Ex. 1044 - AQ*), and *Lo* (1989)(*Ex. 1045 - AR*). Each of these references describes primers that amplify, *e.g.,* genes found on the Y chromosome (*SRY*) and on chromosome 1 (*RHD*). Because these sequences were known to be absent in the mother, these sequences are specific for a fetal nucleic acid. Detection of a Y sequence from a male fetus in a maternal plasma or serum sample using Y-specific primers would identify a male fetus. *Kazakov Decl. at ¶¶ 25-36 and 46* (*Ex. 1046 - AT*).

F. Methods for Determining Concentration of Fetal Nucleic Acid Sequences in a Maternal Sample Were Well Known in 1997

Bianchi, *Mutter*, and *Lo* (1989) each disclose quantitative PCR methods that could be used to determine the concentration of Y chromosome sequences in a maternal plasma or serum sample. *Kazakov Decl. at ¶¶ 38-42* (*Ex. 1046 - AT*). Determining the concentration of the Y chromosome can indicate fetal DNA concentration in two possible ways. First, in a euploid fetus with a normal number of chromosomes, the concentration of Y sequences can be used as a proxy for determining the overall concentration of fetal DNA in a maternal sample (i.e. the "absolute" fetal concentration), as the Y chromosome can only come from the fetus and thus is a unique indicator of fetal DNA in the maternal sample. Second, in a fetus with a Y aneuploidy, detecting the levels of Y sequences in a maternal sample is indicative of the number of additional Y chromosomes in the fetus, and thus the relative concentration of the fetal Y chromosome DNA present in the maternal sample. In either case, detection of increased levels of the Y chromosome in a maternal sample would be indicative of a fetal concentration higher than found in a normal control, as it would indicate an increase in either absolute concentration (in a euploid fetus) or relative concentration (an increased amount of Y chromosome due to the Y aneuploidy). *Id. at ¶¶ 47-51* (*Ex. 1046 - AT*). Using the PCR procedures described by *Bianchi*, *Mutter* or *Lo* (1989), one would have expected to determine either the absolute concentration of fetal nucleic

acids in a maternal plasma or serum sample, or the relative concentration of a particular fetal nucleic acid molecules in a maternal sample from a pregnant woman carrying a male fetus.

G. Detection of Disorders Involving Extra Copies of Chromosomes, such as Down Syndrome

In practice, techniques that merely identify the presence or absence of a nucleic acid were sometimes sufficient to detect a paternally-inherited allele if the nucleic acid sequence is known *a priori* to be absent from the maternal genome. (*Mansfield Decl. at ¶¶ 23-26*) (*Ex. 1047 - AU*). This is because one does not need to know the particular quantity of the nucleic acid with any substantial specificity. (*Mansfield Decl. at ¶ 25*) (*Ex. 1047 - AU*). With respect to such qualitative techniques, the fact that the sequence is present following the amplification tells you what you need to know. (*Mansfield Decl. at ¶ 25*) (*Ex. 1047 - AU*). For example, as in the case of amplification of Y chromosome sequences, if a Y chromosome sequence is present in maternal serum, it could only have originated from a fetal source. (*Mansfield Decl. at ¶¶ 25-26*)(*Ex. 1047 - AU*); *Kazakov Decl. at ¶¶ 46* (*Ex. 1046 - AT*).

Many fetal disorders, however, cannot be detected so simply, such as most common fetal aneuploidies. (*Mansfield Decl. at ¶ 25-26*)(*Ex. 1047 - AU*). Aneuploidy is a condition characterized by an abnormal amount of chromosomal DNA, for example, by an extra or missing chromosome. *Kazakov Decl. at ¶ 54*

(*Ex. 1046 - AT*). Thus, the overall level of fetal DNA in the maternal blood plasma or serum from the aneuploid fetus would be higher or lower than that expected for a normal, non-aneuploid fetus. *Kazakov Decl. at ¶ 60.* For any non-Y aneuploidy, however, detection of an increased relative concentration of the extra fetal chromosome is more difficult due to the background of maternal extracellular DNA present in the maternal sample. *Mansfield Decl. at ¶26.*

Identification of trisomy 21, which is associated with Down syndrome, involves detecting an extra copy of chromosome 21 compared to a non-aneuploid (euploid) fetus. (*Mansfield Decl. at ¶ 26*)(*Ex. 1047 - AU*). The vast majority of cases of trisomy 21 result from the inheritance of two copies of chromosome 21 from the mother and one from the father. *Id*. Thus, to distinguish a fetus with trisomy 21 from a normal euploid fetus containing only two copies of chromosome 21 (one copy from the mother and the other from the father), the test must assess the relative number of maternally and paternally inherited fetal chromosomes to identify the anomaly. *Id*. Trisomy 18, associated with Edwards syndrome, is another example of a disorder that involves an extra copy of a chromosome and thus can be detected only by counting the relative number of occurrences of sequences on chromosome 18. *Id*. Since the mother also has two copies of 21 and 18, and the amount of cell-free DNA in the mother's bloodstream contributed by the fetus is on average about 10%, the amount of fetal DNA that would be

increased in a pregnant woman carrying a fetus with a trisomy 21 or trisomy 18 would be relatively low compared to the overall amounts of the background maternal levels. Detection of such trisomies requires techniques that are sensitive to fairly small increases in levels of DNA for a particular chromosome. Such fine quantitative approaches are needed because of the accuracy of the chromosome counts required to identify the fetal abnormality. *Id*.

Y chromosome aneuploidies, such as XYY syndrome, would likewise be detected by determining the relative number of Y chromosome sequences compared to those from a normal (XY) male fetus. *Id*. One significant difference between detecting a fetal Y-chromosome aneuploidy and other fetal aneuploidies in maternal plasma is that the mother does not possess a Y chromosome, and thus the detection of a Y aneuploidy is not complicated by the presence of maternal DNA having the same genetic composition. Thus, while highly precise methods for quantitation of the fetal extracellular DNA were still needed for the detection of all other aneuploidies, prior art references to the '540 Patent provided a good expectation of determining the fetal condition of aneuploidy of the Y chromosome by detecting an abnormal level of Y chromosome DNA in the maternal plasma or serum. *(Kazakov Decl. at ¶ 51-53 and 55-58* (*Ex. 1046 - AT*)*)*. A difference in levels of Y chromosome in the serum or plasma of a woman carrying an XYY fetus compared to the level found in women carrying a normal, non-aneuploid

fetus would need to be essentially on the order of a 2:1 difference in DNA levels, versus a difference of 1.1 to 1.15 for other aneuploidies in which the fetal DNA contribution in a maternal sample is 10%. (*Mansfield Decl. at ¶ 26*)(*Ex. 1047 - AU*). For a fetus that is XYYY, the difference in levels of Y chromosome in the maternal serum or plasma is even larger, 3:1 versus a euploid XY fetus. (*Mansfield Decl. at ¶ 125*)(*Ex. 1047 - AU*). Thus, even with the techniques available in 1998, a Y chromosome aneuploidy could in some instances be detected because the differences in the magnitude of Y chromosome sequences present in maternal serum or plasma of a woman carrying a male fetus with a Y-chromosome aneuploidy were significant compared to the levels of the Y chromosome in a maternal sample from a woman carrying a euploid male fetus. The declaration of Dr. Mansfield explains why most fetal copy number variants (*e.g.*, trisomies or translocations) and monogenic diseases of the fetus could not be quantitatively analyzed based on the disclosure of the '540 Patent. (*Mansfield Decl. at ¶¶ 24-26*) (*Ex. 1047 - AU*). She also discloses why the prior art references could have enabled detection of certain Y aneuploidies. (*Mansfield Decl. at ¶¶ 27-39*) (*Ex. 1047 - AU*).

VI. BROADEST REASONABLE CONSTRUCTION

Petitioner bases the instant petition upon the broadest reasonable interpretation of the claim language. Petitioner's position regarding the scope of the claims under their broadest reasonable interpretation is not to be taken as stating any position regarding the appropriate scope to be given the claims in a court or other adjudicative body under the different claim interpretation standards which apply in such proceedings.

Some of the terms in claims 1-3, 5, 12, 13, 15, and 18 were interpreted in the Decision. In the Decision it was stated that "DNA of fetal origin that could be detected using the disclosed methods in view of the Specification" was interpreted "as DNA from a fetus from 7 to 40 weeks of gestation." Based on the foregoing, the terms "**fetal**" and "**fetus**" as used in claims 1-3, 5, 12, 13, 15, and 18 include a fetus from 7 to 40 weeks of gestation.

In claim 1, the step "**detecting the presence of a paternally inherited nucleic acid from the serum or plasma sample**" was interpreted as not requiring "that the nucleic acid be specifically identified as being inherited from the father or even as being from the fetus, only that it be identified as containing some level of nucleic acid." It was further noted in the Decision that Dr. Evans stated that "the ordinary artisan would interpret 'detect' as only requiring 'discovery or determining the existence, presence, or fact of,' thus determining the presence of

fetal nucleic acid does not require distinguishing it from maternal nucleic acid."

Claim 5 recites detection of "**the presence of a fetal nucleic acid sequence from the Y chromosome,**" which under the broadest reasonable interpretation includes discovery of or determining the existence, presence, or fact of DNA amplified by Y chromosome primers, which also permits amplified male fetal DNA to be distinguished from the maternal DNA due to the absence of the Y chromosome in the maternal DNA. *Kazakov Decl. at ¶¶ 46* (*Ex. 1046 - AT*); (*Mansfield Decl. at ¶ 69*) (*Ex. 1047 - AU*).

The term "**concentration**" is used in claim 13 regarding "the concentration of fetal nucleic acid sequence in the maternal serum or plasma." The '540 Patent (*Ex. 1001 – A*) does not explain the meaning of "concentration" and has various references to mean concentration (*col. 15, lines 38-41*), absolute concentration (*col. 15, lines 41-43*), fractional concentration (*col. 15, lines 43-46*), and relative concentration (*col. 16, lines 21-22*). Petitioner proposes that the broadest reasonable interpretation of this term encompasses the amount of fetal nucleic acid sequence compared to the maternal serum or plasma as described by Dr. Mansfield. (*Mansfield Decl. at ¶¶ 103-106*) (*Ex. 1047 - AU*). Further, because claim 13 depends from claim 5 and based on the antecedent basis for "the foetal nucleic acid sequence" recited in claim 13, the term concentration is understood to refer to the concentration of the fetal nucleic acid sequence as determined by

detection of the Y chromosome.[1]

The term "**normal**" is used in claim 15 within the phrase "the level of foetal DNA in the maternal serum or plasma is higher or lower than normal." This phrase lacks antecedent basis in claim 13 from which claim 15 depends and the meaning of the term "normal" is not specified. Petitioner proposes that the broadest reasonable interpretation of the word "normal" refers to the level of extracellular fetal DNA of a euploid fetus typically found in a maternal plasma or serum sample. (*Mansfield Decl. at ¶¶ 119-121*)(*Ex. 1047 - AU*). Factors which may affect levels of fetal DNA in a maternal sample include gestational age, sex, innate biological variability, cytogenetic status, aneuploid conditions, and fetal, biological or immunological incompatibilities with the mother. This view is consistent with *Simpson*, which describes maternal and fetal conditions in which the level of fetal DNA in the serum or plasma would be higher or lower than normal. In particular, it is stated in *Simpson* that: "The frequency doubtless

[1] There is no express description of determining fetal DNA concentration in Great Britain Patent Application No. 9,704,444 (Ex. 1004 – D), which uses the word "concentration" only on page 6, line 2, wherein it is stated that "[i]t is likely that placental damage in pre-eclampsia may result in alterations in foetal DNA concentration in material serum and plasma." The '540 patent is not entitled to its claimed priority date for this additional reason. (See *Mansfield Decl. at ¶107*, *Ex. 1047 – AU, and the cases cited in Section V.B. of the 1st petition)*

reflects many factors, particularly gestational age, innate biological variability and cytogenetic status of the pregnancy (*Ex. 1025 - X, p. 1236, 1st full para*). Under a broad interpretation of the term "normal" this reference term excludes variations in fetal DNA content caused by any genetic differences or conditions described by *Simpson* including Mendelian disorders, gender differences, RhD or ABO incompatibilities with the mother. (*Ex. 1025 - X, p. 1233, col. 2 to p. 1234, col. 2 & p. 1237, cols. 1-2*). (*Mansfield Decl. at ¶ 119*) (*Ex. 1047 - AU*). The term "normal" also explicitly excludes aneuploidies, which by definition increase the concentration of fetal DNA relative to a "normal" euploid fetus. *Supra.*

Claim 18 recites "[t]he method according to claim 13, **for detection of a foetal chromosomal aneuploidy**." Because claim 18 depends from claim 13, the phrase in claim 18 is a further limitation of the scope of claim 13, which recites "[t]he method according to claim 5, which comprises determining the concentration of the foetal nucleic acid sequence in the maternal serum or plasma." Additionally, because claim 13 depends from claim 5, the fetal nucleic acid sequence recited in claim 13 is referring to a fetal nucleic acid sequence from the Y chromosome. Reading these claims together, it is clear that the "detection of a foetal chromosomal aneuploidy" recited in claim 18 is achieved by determining the concentration of the fetal nucleic acid sequence from the Y chromosome in the maternal serum or plasma. Dr. Mansfield interprets claim 18 to mean that

"detection of a foetal chromosomal aneuploidy" constitutes "detecting an elevated level of fetal DNA indicative of an increased copy number of a fetal chromosome." (*Mansfield Decl. at ¶ 124*) (*Ex. 1047 - AU*). Given the dependency of claim 18 from claims 5 and 13, it is proper to understand claim 18 as referring to an increased level of Y sequences indicative of Y-chromosome aneuploidy, or alternatively an increased level of Y sequences indicative of an increase in absolute concentration of fetal DNA based due to the presence of a non-Y fetal chromosome aneuploidy. Based on the foregoing, petitioner proposes that the broadest reasonable interpretation of claim 18 is detection of a fetal chromosome aneuploidy based on a determination of an increased concentration of Y-chromosome fetal nucleic acid sequences in the maternal serum or plasma. (*Id.)*

VII. REPRESENTATIVE PROPOSED REJECTIONS AND SHOWING THAT PETITIONER IS LIKELY TO PREVAIL

The references addressed below each variously anticipate or render obvious the claimed subject matter.

A. Claims 3, 12, 13, 15 and 18 Are Rendered Obvious by *Kazakov* Taken in Combination with *Bianchi*

The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is anticipated by *Kazakov* (*Ex. 1014 - N*). (*Decision, pp. 19-25, and 37, Ex. 1042 - AN*). The claim chart below sets forth the correspondence between the claims of the '540 Patent and the combined teachings of *Kazakov* (*Ex. 1014 -N*) and *Bianchi* (*Ex. 1043 - AP*). As shown below, claims 3, 12, 13, 15 and 18 are rendered obvious by *Kazakov* in light of *Bianchi*.

US 6,258,540 Claim Language	**Correspondence to *Kazakov* (*Ex. 1014 - N*) and *Bianchi* (*Ex. 1043 - AP*)**
1. A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises amplifying a paternally inherited	The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is anticipated by *Kazakov*. (*Decision, pp. 19-25, and 37, Ex. 1042 - AN*). The corresponding findings in the Decision are incorporated herein by reference. The original declarations of Barker, Kazakov, Mansfield, and Vasioukhin, submitted herewith as Exhibits 1005-1008, set forth a *prima facie* case of unpatentability for claim 1 for the same reasons detailed in the 1st petition.

nucleic acid from the serum or plasma sample and detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample.	
2. The method according to claim 1, wherein the foetal nucleic acid is amplified by the polymerase chain reaction.	*Kazakov*, p. 233, "Material and Method" describes using PCR to amplify Alu and inter-Alu sequences present in maternal serum or plasma. *Kazakov* inherently amplifies fetal Alu and inter-Alu sequences since such sequences are ubiquitous in both fetal and maternal genomes. *See also Kazakov Decl. at ¶¶ 9-36* (*Ex. 1046 - AT*). *Bianchi* teaches PCR for amplifying fetal DNA in a sample of mixed maternal and fetal DNA obtained from isolated blood cells: STUDY DESIGN: Samples from 40 pregnant women were flow sorted with different monoclonal antibodies. Deoxyribonucleic acid was subsequently purified from candidate fetal cells; polymerase chain reaction was performed with synthetic primers specific for sequences on chromosomes Y and 7. (*Ex. 1043 - AP, abstract*). *See Kazakov Decl. at ¶¶ 9-36* (*Ex. 1046 - AT*) and (*Mansfield Decl. at ¶¶ 132*)(*Ex. 1047 - AU*).
3. The method according to claim 2, wherein at least one foetal sequence specific oligonucleotide primer is used in the amplification.	*Kazakov* uses primers that amplify paternally inherited fetal Alu and inter-Alu sequences in a maternal plasma or serum sample. *(See discussion above in connection with claim 2).* *Bianchi* teaches at least one fetal sequence specific oligonucleotide primer since her Y chromosome primers are specific for Y chromosome DNA in a male fetus, but absent from the mother.

5. The method according to claim 1, wherein the presence of a foetal nucleic acid sequence from the Y chromosome is detected.	> Male fetal cell-specific polymerase chain reaction reactions were set up with DNA primers that are complementary to a sequence, on the Y chromosome, Y49a. (*Bianchi*, *Ex. 1043 - AP*, p. 923, col. 2, last para); *see also Kazakov Decl. at ¶¶ 37-39* (*Ex. 1046 - AT*). As explained in the declarations of Kazakov and Mansfield, it would have been obvious to substitute the primers of *Bianchi* for the Alu and inter-Alu primers of *Kazakov*. *See Kazakov Decl. at ¶¶ 9-32, 36-37, 45* (*Ex. 1046 - AT*) and (*Mansfield Decl. at ¶¶ 132-133*)(*Ex. 1047 - AU*).
12. The method according to claim 5, for determining the sex of the foetus.	*See* remarks for claims 3 and 5 above; *see also Kazakov Decl. at ¶¶ 46* (*Ex. 1046 - AT*). As explained in the declarations of Kazakov and Mansfield, it would have been obvious to use the Y chromosome fetal sequence specific primers of *Bianchi* to amplify fetal DNA of paternal origin in a male fetus for the purpose of identifying the sex of a male fetus. *See Kazakov Decl. at ¶¶ 9-30 and 46* (*Ex. 1046 - AT*) and (*Mansfield Decl. at ¶ 30*)(*Ex. 1047 - AU*). This would by definition determine the sex of the fetus, as detection of the Y chromosome is consistent with a male fetus.
13. The method according to claim 5, which comprises determining the concentration of the foetal nucleic acid sequence in the maternal serum or plasma.	In the combined method the concentration of fetal nucleic acid sequence from the Y chromosome is measured according to the technique described in *Bianchi*. *See Kazakov Decl. at ¶¶ 47, 48 and 55-56* (*Ex. 1046 - AT*) and *Mansfield Decl. at ¶¶ 135*)(*Ex. 1047 - AU*). *Bianchi* uses quantitative PCR to determine a ratio of (fetal DNA) to (fetal DNA + maternal DNA), which is the relative concentration of fetal DNA in the DNA sample. *Kazakov Decl. at ¶¶ 47, 48, and 55-56* (*Ex. 1046 - AT*). *Bianchi* quantitates and compares the amount of amplified Y chromosome DNA and the amount of amplified Chromosome DNA as follows: > Gel analysis and quantitation. Amplified products were loaded onto 2% Seakem ME agarose gels (FMC Bioproducts, Rockland, Me.) and separated by electrophoresis at a constant current (175 mA)

	for 1.5 hours. The gels were subsequently dried and exposed to a phosphorimager (Molecular Dynamics, Sunnyvale, Calif.). For each product of the amplification reaction the counts per minute were determined. Male specific fetal cell numbers and total cell numbers were calculated by comparing the degree of label incorporated into sample-specific polymerase chain reaction products with Y-specific and D9-specific standard curves generated independently for every analysis. For a permanent record the dried gel was also exposed to x-ray film (Kodak X-omat, Eastman Kodak, Rochester, N.Y.) at -80°C with intensifying screens for 2 hours. (*Ex. 1043 - AP*, p. 924, col. 1, last para)
15. The method according to claim 13, for the detection of a maternal or foetal condition in which the level of foetal DNA in the maternal serum or plasma is higher or lower than normal.	*Kazakov* teaches that cell death is associated with elevated levels of cell-free DNA in maternal plasma or serum. (*Kazakov*, *Ex. 1014 -N*, p. 233, bottom of first full para). This suggests that conditions exhibiting abnormal cellular proliferation, differentiation and cell death would be characterized by abnormal serum or plasma DNA levels. *Kazakov Decl. at ¶¶ 53-54* (*Ex. 1046 - AT*); (*Mansfield Decl. at ¶¶ 116, 119-123*)(*Ex. 1047 - AU*). As explained in the declarations of Kazakov and Mansfield, a skilled artisan would have been motivated to determine the concentration of fetal DNA in a maternal serum or plasma sample using the methods of *Bianchi* and compare that to a "normal" value for the purpose of detecting a maternal or fetal condition characterized by a higher or lower level fetal DNA in the serum or plasma. *See Kazakov Decl. at ¶¶ 52-54* (*Ex. 1046 - AT*) and (*Mansfield Decl. at ¶ 136*)(*Ex. 1047 - AU*). *Bianchi* describes detection of fetal aneuploidy, which is a condition in which the relative concentration of a particular chromosome would be elevated compared to a non-aneuploid state. (*Ex. 1043 - AP*, Abstract and p. 922, col. 1, first para). As explained in the Declaration of Professor Kazakov, this

	provides further motivation for determining the concentration of fetal DNA in plasma or serum relative to a normal, non-aneuploid pregnancy at the same stage of gestation. *Kazakov Decl. at ¶¶ 52-54* (*Ex. 1046 - AT*).
18. The method according to claim 13, for detection of a foetal chromosomal aneuploidy.	*Bianchi* describes the detection of an aneuploid condition: > The existence and persistence of fetal cells in the maternal circulation is no longer considered controversial. The detection of fetal aneuploidy, fetal gene mutations, fetal polymorphisms, and fetal gender by many investigators working independently has demonstrated the feasibility of prenatal genetic diagnosis by maternal venipuncture. (*Bianchi*, *Ex. 1043 - AP*, p. 922, 1st paragraph) As discussed above in connection with claims 2, 3 and 5, *Kazakov* inherently amplified paternally inherited Alu and inter-Alu sequences, including those on the Y chromosome, and indicated that cell free DNA in maternal serum could only have two sources--the fetus and the mother. The declarations of Kazakov and Mansfield explain that it would have been obvious in view of *Kazakov* and *Bianchi* to detect a fetal aneuploidy by detecting a lower or higher than normal concentration of fetal DNA from the Y chromosome of a fetus compared to normalized levels from an XY fetus. *See Kazakov Decl. at ¶¶ 52-54* (*Ex. 1046 - AT*) and (*Mansfield Decl. at ¶ 137*)(*Ex. 1047 - AU*).

B. Claims 3, 12, 13, 15 and 18 Are Rendered Obvious by *Kazakov* Taken in Combination with *Mutter*

The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is anticipated by *Kazakov*. (*Decision, pp. 19-25, and 37, Ex. 1042 - AN*). The claim chart below sets forth the correspondence between the claims of the '540 Patent and the combined teachings of *Kazakov* (*Ex. 1014 - N*) and *Mutter* (*Ex. 1048 - AS*). As shown below, claims 3, 12, 13, 15 and 18 are rendered obvious by *Kazakov* in light of *Mutter*.

US 6,258,540 Claim Language	**Correspondence to *Kazakov* (*Ex. 1014 - N*) and *Mutter* (*Ex. 1048 - AS*)**
1. A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises amplifying a paternally inherited nucleic acid from the serum or plasma sample and detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample.	The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is anticipated by *Kazakov*. (*Decision, pp. 19-25, and 37, Ex. 1042 - AN*). The corresponding findings in the Decision are incorporated herein by reference. The original declarations of Barker, Kazakov, Mansfield, and Vasioukhin, submitted herewith as Exhibits 1005-1008, set forth a *prima facie* case of unpatentability for claim 1 for the same reasons detailed in the 1st petition.
2. The method according to claim 1, wherein the foetal	*Kazakov*, p. 233, "Material and Method" describes using PCR to amplify Alu and inter-Alu sequences present in maternal serum or plasma. *Kazakov* inherently amplifies fetal Alu and

nucleic acid is amplified by the polymerase chain reaction.	inter-Alu sequences since such sequences are ubiquitous in both fetal and maternal genomes. *See also Kazakov Decl. at ¶¶ 9-30* (*Ex. 1046 - AT*). *Mutter* teaches amplifying Y chromosome DNA containing the Y chromosome marker ZFY (*Mutter*, *Ex. 1048 - AS*, Abstract, p. 4204, "PCR Amplification"). As explained in the declarations of Kazakov and Mansfield, it would have been obvious to use PCR to amplify paternally inherited nucleic acid in a maternal plasma or serum sample in view of *Kazakov* and *Mutter*. *See Kazakov Decl. at ¶¶ 35, 37, 38, 40 and 45* (*Ex. 1046 - AT*) and (*Mansfield Decl. at ¶ 139*)(*Ex. 1047 - AU*).
3. The method according to claim 2, wherein at least one foetal sequence specific oligonucleotide primer is used in the amplification. 5. The method according to claim 1, wherein the presence of a foetal nucleic acid sequence from the Y chromosome is detected."	*Kazakov* uses primers that amplify paternally inherited fetal Alu and inter-Alu sequences in a maternal plasma or serum sample. *(See discussion above in connection with claim 2).* *Mutter* teaches X and Y chromosome specific primers (*Mutter*, *Ex. 1048 - AS*, abstract, p. 4204, "PCR Amplification"); *see also Kazakov Decl. at ¶¶ 35, 37, 38, 40, 45* and 46 (*Ex. 1046 - AT*). As explained in the declarations of Kazakov and Mansfield, it would have been obvious to substitute the Y chromosome primers described by *Mutter* for the Alu and inter-Alu primers of *Kazakov*. *See Kazakov Decl. at ¶¶ 9-30, 35, 37, 38, 40, 45* and 46 (*Ex. 1046 - AT*); (*Mansfield Decl. at ¶¶ 139-140*)(*Ex. 1047 - AU*).
12. The method according to claim 5, for determining the sex of the foetus.	*See* remarks for claims 3 and 5 above; *see also Kazakov Decl. at ¶¶ 46* (*Ex. 1046 - AT*). As explained in the declarations of Kazakov and Mansfield, it would have been obvious to use the Y chromosome fetal sequence specific primers of *Mutter* to amplify fetal DNA of paternal origin in a male fetus for the purpose of identifying the sex of a male fetus. *See Kazakov Decl. at ¶¶ 9-30 and 46* (*Ex. 1046 - AT*); (*Mansfield Decl. at ¶ 30*)(*Ex. 1047 - AU*).
13. The method according to claim 5,	In the combined method of *Kazakov* and *Mutter*, the concentration of foetal nucleic acid sequence from the Y

which comprises determining the concentration of the foetal nucleic acid sequence in the maternal serum or plasma.	chromosome is measured according to the technique described in *Mutter*. *See Kazakov Decl. at ¶¶ 47 and 49* (*Ex. 1046 - AT*); (*Mansfield Decl. at ¶ 142*)(*Ex. 1047 – AU*). *Mutter* teaches a method for determining the relative concentration of nucleic acid sequences from the X chromosome and from the Y chromosome. (*Mutter*, *Ex. 1048 - AS*, abstract, "PCR Amplification" and "Densitometry", pp. 4204-05). Alternatively, if claim 13 is more broadly interpreted to cover determining the concentration of the total fetal DNA in the maternal serum or plasma, it would be obvious to calculate the absolute concentration of fetal DNA in the sample by simply multiplying the relative concentration of fetal DNA (i.e., the percentage of fetal DNA in the sample) by the total concentration of DNA in the DNA sample. (*Mansfield Decl. at ¶ 142*)(*Ex. 1047 – AU*).
15. The method according to claim 13, for the detection of a maternal or foetal condition in which the level of foetal DNA in the maternal serum or plasma is higher or lower than normal. 18. The method according to claim 13, for detection of a foetal chromosomal aneuploidy.	*Kazakov* teaches that cell death is associated with elevated levels of cell-free DNA in maternal plasma or serum. (*Kazakov*, *Ex. 1014 -N*, p. 233, bottom of first full para). This suggests that conditions exhibiting abnormal cellular proliferation, differentiation and cell death would be characterized by abnormal serum or plasma DNA levels. *Kazakov Decl. at ¶¶ 52 and 53* (*Ex. 1046 - AT*); (*Mansfield Decl. at ¶¶ 119-123*)(*Ex. 1047 - AU*). The term "normal" encompasses variations in fetal DNA content caused by the genetic differences or conditions described by *Mutter* including Turner syndrome, Kleinfelter syndrome and other abnormal sex chromosome genotypes (*Mutter*, *Ex. 1048 - AS*, p. 4203, col. 2, 1st para) as well as abnormal conceptuses (p. 4207, 2nd full para). Thus, *Mutter* describes maternal and fetal conditions in which the level of fetal DNA in the serum or plasma would be higher or lower than normal. As explained in the declarations of Kazakov and Mansfield, one of ordinary skill in the art would have been motivated to determine the concentration of fetal DNA in a maternal serum or plasma sample using the methods of *Mutter* and compare that to a "normal" value for the purpose of detecting a maternal

	or fetal condition characterized by a higher or lower level of fetal DNA in the serum or plasma. *See Kazakov Decl. at ¶¶ 51-57* (*Ex. 1046 - AT*) and (*Mansfield Decl. at ¶¶ 143*)(*Ex. 1047 - AU*). *Mutter* also provides motivation to detect a fetal aneuploidy by detecting a lower or higher than normal concentration of fetal DNA from a putative aneuploid chromosome and a non-aneuploid chromosome. *See Kazakov Decl. at ¶¶ 51-58* (*Ex. 1046 - AT*) and (*Mansfield Decl. at ¶¶ 144*)(*Ex. 1047 - AU*).

C. Claims 3, 12, 13, 15 and 18 Are Anticipated by Lo (1997)

The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is anticipated by *Lo* (1997) (*Ex. 1016 - P*). (*Decision, pp. 19-25, and 37, Ex. 1042 - AN*). The claim chart below sets forth the correspondence between Lo (1997) and the claims of the '540 Patent. As demonstrated therein, claims 3, 12, 13, 15 and 18 are also anticipated by the Lo (1997) reference.

US 6,258,540 Claim Language	**Correspondence to *Lo* (1997)(Ex. *1016 - P*)**
1. A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises amplifying a paternally inherited nucleic acid from the serum or plasma sample and detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample.	The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is anticipated by *Lo* (1997) (*Ex. 1016 - P*). (*Decision, pp. 15-16, and 37, Ex. 1042 - AN*). The corresponding findings in the Decision are incorporated herein by reference. The original declarations of Barker, Kazakov, Mansfield, and Vasioukhin, submitted herewith as Exhibits 1005-1008, set forth a *prima facie* case of unpatentability for claim 1 for the same reasons detailed in the 1st petition.
2. The method according to claim 1, wherein the foetal nucleic acid is	*Lo* (1997) discloses amplification of fetal sequence specific oligonucleotide primers Y1-7 and Y1-S by PCR: *PCR*

amplified by the polymerase chain reaction. 3. The method according to claim 2, wherein at least one foetal sequence specific oligonucleotide primer is used in the amplification.	The PCR was carried out broadly as described elsewhere with reagents from a GeneAmp DNA Amplification Kit (Perkin Elmer, Foster City, CA, USA). The detection of Y-specific fetal sequence from maternal plasma, serum, and cellular DNA was done as described with primers Y1-7 and Y1-S, designed to amplify a single-copy sequence (DYS14). The Y-specific product was 198 bp. 60 cycles of Hot Start PCR with Ampliwax technology were used on 10 µl maternal plasma or serum, or on 100 ng maternal nucleated blood-cell DNA; each cycle consists of a denaturation step at 94°C for 1 min, and a combined reannealing/ extension step at 57°C for 1 min. 40 cycles were used for amplification of amniotic fluid. PCR products were analysed by agarose-gel electrophoresis and ethidium-bromide staining. PCR results were scored before fetal sex was revealed to the investigator. (*Lo* (1997), *Ex. 1016 - P*, p. 486, col. 1, 2nd paragraph); *see also Kazakov Decl. at ¶¶ 43, 66 and 69* (*Ex. 1046 - AT*). Primers Y1-7 and Y1-8 are "foetal sequence specific oligonucleotide primers" used in the amplification of fetal DNA from a male fetus. *Kazakov Decl. at ¶ 43, 66, 69 and 71* (*Ex. 1046 - AT*). Y chromosome specific primers would not amplify maternal DNA because the mother carries two X chromosomes and does not have a Y chromosome. *Id*.
5. The method according to claim 1, wherein the presence of a foetal nucleic acid sequence from the Y chromosome is detected.	*See* remarks for claim 3; *see also Kazakov Decl. at ¶ 46* (*Ex. 1046 - AT*). For a male fetus, the *Lo* Primers Y1-7 and Y1-8 amplify and detect "a foetal nucleic acid sequence from the Y chromosome." *Kazakov Decl. at ¶¶ 43-44* (*Ex. 1046 - AT*).
12. The method according to claim 5, for determining the sex of the foetus.	*See* remarks for claims 3 and 5; *see also Kazakov Decl. at ¶¶ 46, 75* (*Ex. 1046 - AT*). Lo teaches determining the sex of the fetus:

	Maternal plasma and serum samples were collected from 43 women who were between 12 and 40 weeks pregnant. There were 30 male and 13 female fetuses. Among the 30 women bearing male fetuses, Y-positive signals were detected in 24 plasma samples and 21 serum samples when 10 µL of the samples was used for PCR (figure and table). When DNA from nucleated blood-cells was used for Y-PCR, positive signals were detected in only five of the 30 cases (table). None of the 13 women bearing female fetuses, and none of the ten non-pregnant control women, had a positive Y signal when plasma, serum, or cellular DNA was amplified. (*Lo* (1997), *Ex. 1016 - P*, p. 486, col. 1, para 5) The method of *Lo* can be used "for determining the sex of the foetus" because the sex of the fetus would be determined by the detection of amplified Y chromosome DNA. As is apparent from the paragraph above, *Lo* exemplifies determination of the sex of male fetuses.
13. The method according to claim 5, which comprises determining the concentration of the foetal nucleic acid sequence in the maternal serum or plasma.	*Lo* measures the relative fetal DNA concentration based on the stage of gestation as well as determines a functional concentration of fetal DNA sufficient to permit detection of the fetal Y chromosome: **In four cases with male fetuses, both plasma and serum were negative for fetal DNA (cases 1, 11, 12, and 30). Three of these women were tested at 15 weeks of pregnancy or earlier (cases 1, 11, and 12).** Furthermore, of the seven cases in which there was discordance between plasma and serum samples, all but one were tested before 23 weeks. Taken together, these results suggest that the **concentration of fetal DNA increases as gestation progresses**, possibly owing to the increase in fetal size. This result is analogous to that of Nawroz and colleagues, who detected in head and neck cancer patients mutant plasma DNA predominantly in those with high tumour load. Future studies should investigate the

	temporal relation between gestation and the appearance and concentration of fetal DNA in maternal plasma. (*Lo* (1997), *Ex. 1016 - P*, p. 487, col. 1, penultimate full paragraph). This approach allows us to compare the relative detectability of fetal DNA in 10 µL plasma or serum and the cellular component of 10 µL whole blood. The detection rate of fetal DNA in 10 µL plasma and serum is already high at 80% and 70%, respectively, but these rates can probably be improved. (*Lo* (1997), *Ex. 1016 - P*, p. 487, col. 1, lines 5-10). This disclosure shows that *Lo* determined a functional concentration of fetal DNA in maternal plasma or serum. *Kazakov Decl. at ¶ 76* (*Ex. 1046 - AT*).
15. The method according to claim 13, for the detection of a maternal or foetal condition in which the level of foetal DNA in the maternal serum or plasma is higher or lower than normal. 18. The method according to claim 13, for detection of a foetal chromosomal aneuploidy.	See remarks for claim 13. *Lo* (1997) found that some maternal serum samples contained no fetal DNA thus determining a concentration of 0% fetal DNA: "In four cases with male fetuses, both plasma and serum were negative for fetal DNA (cases 1, 11, 12, and 30)" (*Lo* (1997), *Ex. 1016 - P*, p. 487, col. 1, 2nd to last para). *Lo* (1997) describes a method for determining fetal DNA concentration in a sample: "Serial dilutions of male genomic DNA in 1 µg female genomic DNA were carried out and amplified by the Y-PCR system with 60 cycles of amplification. Positive signals were detected up to the 1/100000 dilution-i.e., the approximate equivalent of a single male cell." (Lo, *Ex. 1016 - P*, p. 486, col. 1, 3rd paragraph) *Lo* (1997) states that "Taken together, these results suggest that the concentration of fetal DNA increases as gestation progresses, possibly owing to the increase in fetal size…." and "The plasma or serum-based approach might also be applicable

	to screening for chromosomal aneuploidies (such as Down's syndrome) if there is a quantitative difference in the concentration of fetal DNA in maternal plasma and serum between affected and normal pregnancies; this is a situation analogous to the high concentration of fetal cells detectable in pregnancies that involve aneuploid fetuses" (*Lo* (1997), *Ex. 1016 - P*, p. 487, col. 1, last two full paragraphs)

D. Claims 3, 12, 13, 15 and 18 Are Rendered Obvious by *Lo* (1997) Taken in Combination with *Lo* (1990) and *Lo* (1989)

The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is anticipated by *Lo* (1997) (*Ex. 1016 - P*). (*Decision, pp. 15-16, and 37, Ex. 1042 - AN*). The claim chart below sets forth the correspondence between the claims of the '540 Patent and the combined teachings of *Lo* (1997) (*Ex. 1016 - P*), *Lo* (1990) (*Ex. 1044 - AQ*) and *Lo* (1989) (*Ex. 1045 - AR*). As shown below, claims 3, 12, 13, 15 and 18 are rendered obvious by *Lo* (1997) taken in combination with *Lo* (1990) and *Lo* (1989).

US 6,258,540 Claim Language	**Correspondence to *Lo* (1997)(Ex. *1016 - P*), *Lo* (1990) (Ex. 1044 - AQ), and *Lo* (1989) (Ex. 1045 - AR)**
1. A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises amplifying a paternally inherited nucleic acid from the serum or plasma sample and detecting the presence of a	The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is anticipated by *Lo* (1997) (*Ex. 1016 - P*). (*Decision, pp. 15-16, and 37, Ex. 1042 - AN*). The corresponding findings in the Decision are incorporated herein by reference. The original declarations of Barker, Kazakov, Mansfield, and Vasioukhin, submitted herewith as Exhibits 1005-1008, set forth a *prima facie* case of unpatentability for claim 1 for the same reasons detailed in the 1st petition.

paternally inherited nucleic acid of fetal origin in the sample.	
2. The method according to claim 1, wherein the foetal nucleic acid is amplified by the polymerase chain reaction.	*Lo* (1989) describes extracting a repeating portion of Y chromosome DNA and amplifying it using polymerase chain reaction (PCR): > We used PCR to determine fetal sex from maternal peripheral blood, with the correct identification in all pregnancies, at 9-41 weeks' gestation. The second round of amplification gave the added sensitivity essential for success. Use of extracted DNA, rather than whole cells, may also increase sensitivity because the Y-specific sequence chosen is present as a tandem array of 800-5000 subunits[17] which is broken up into much smaller subunits and dispersed into solution and thoroughly mixed during the extraction procedure. (*Ex. 1045 - AR* at pp. 1364-65). The *Lo* PCR procedure exhibited high sensitivity by detecting target DNA in a 10,000,000-fold dilution of male DNA into female DNA. > 10-fold serial dilutions of male DNA in female DNA were performed to determine the sensitivity of the dual amplification system. After the first round of amplification, positive bands were present at reciprocal dilutions of 10^4-10^5. After the second round of PCR with internal primers Y1.3 and Y1.4 (15 cycles), the detection limit was extended to 1 in 10^7. (*Ex. 1045 - AR* at p. 1364, bottom of col. 2). *Lo* (1990) describes extracting single copy Y chromosome DNA, which is paternally inherited nucleic acid, and amplifying it using polymerase chain reaction. > We have reported amplification of a Y-specific repeat sequence from the peripheral blood of

	women pregnant with male fetuses[1] by use of the polymerase chain reaction (PCR).[2] However, before the method can be applied to prenatal diagnosis of non-sex-linked genetic disorders it is essential that single-copy fetal sequences be amplified from maternal blood. We now report the amplification of a single-copy Y-specific sequence from peripheral blood DNA of pregnant women. *(Ex. 1044 - AQ at pp. 1463, 1st para).*
3. The method according to claim 2, wherein at least one foetal sequence specific oligonucleotide primer is used in the amplification. *5. The method according to claim 1, wherein the presence of a foetal nucleic acid sequence from the Y chromosome is detected.* 12. The method according to claim 5, for determining the sex of the foetus.	*Lo* (1989) teaches male fetal specific primers 1-1 to 1-4 (Ex. 10A, p. 1364, paragraph bridging cols. 1-2). The primers are specific for a male foetus because maternal DNA in a mixed maternal and male fetus DNA sample would not contain Y chromosome sequences. *Lo* (1990) also discloses fetal sequence specific primers: We now report the amplification of a single-copy Y-specific sequence from peripheral blood DNA of pregnant women. . . . The new primer sequences are: Yl-5 = CTAGACCGCAGAGGCGCCAT Yl-6 = TAGTACCCACGCCTGCTCCGG Yl-7 = CATCCAGAGCGTCCCTGGCTT Yl -8 = CTTTCCACAGCCACATTTGTC. (*Lo* (1990), *Ex. 1044 - AQ*, p. 1463, bottom of col. 1) One would have been motivated to perform PCR using a fetal sequence specific primer such as the Y chromosome primers of *Lo* (1997), *Lo* (1990), or *Lo* (1989) in order to detect a paternally inherited Y chromosome sequence that would identify the male sex of a foetus. *Kazakov Decl. at ¶ 75* (*Ex. 1046 - AT*).
13. The method according to claim 5, which comprises determining the concentration of the	*Lo* (1990) reports the detection of the relative concentration of amplified Y chromosome DNA to that of a co-amplified α-antitrypsin nucleic acid sequence thus showing that the Y sequence that was amplified was a single copy sequence. (*Lo* (1990, *Ex. 1044 - AQ*, p. 1463, col. 1, last para)

foetal nucleic acid sequence in the maternal serum or plasma.	
15. The method according to claim 13, for the detection of a maternal or foetal condition in which the level of foetal DNA in the maternal serum or plasma is higher or lower than normal. 18. The method according to claim 13, for detection of a foetal chromosomal aneuploidy.	See remarks for claim 13. *Lo* (1997) found that some maternal serum samples contained no fetal DNA thus determining a concentration of 0% fetal DNA: "In four cases with male fetuses, both plasma and serum were negative for fetal DNA (cases 1, 11, 12, and 30)" (*Lo* (1997), *Ex. 1016 - P*, p. 487, col. 1, 2nd to last full para). *Lo* (1997) describes a method for determining fetal DNA concentration in a sample: "Serial dilutions of male genomic DNA in 1 µg female genomic DNA were carried out and amplified by the Y-PCR system with 60 cycles of amplification. Positive signals were detected up to the 1/100000 dilution-i.e., the approximate equivalent of a single male cell." (Lo, *Ex. 1016 - P*, p. 486, col. 1, 3rd paragraph) *Lo* (1997) states that "Taken together, these results suggest that the concentration of fetal DNA increases as gestation progresses, possibly owing to the increase in fetal size…." and "The plasma or serum-based approach might also be applicable to screening for chromosomal aneuploidies (such as Down's syndrome) if there is a quantitative difference in the concentration of fetal DNA in maternal plasma and serum between affected and normal pregnancies; this is a situation analogous to the high concentration of fetal cells detectable in pregnancies that involve aneuploid fetuses" (*Lo* (1997), *Ex. 1016 - P*, p. 487, col. 1, last two full paragraphs) Alternatively, should "detection of a foetal chromosomal aneuploidy" be considered to include detection of fetal aneuploidy by determining abnormal ratios of different fetal DNA molecules, *Lo* (1990) shows that PCR amplification of fetal DNA can detect single gene copies and that gene copy in a DNA sample can be determined by comparing genes on different chromosomes, namely the Y chromosome and the chromosome containing the α-antitrypsin gene, to determine

	copy number of a particular gene. As explained in the Declaration of Professor Kazakov, *Lo* (1990) discloses a co-amplification method that: > could also have been used to determine the relative amount of Y chromosome DNA in a sample compared to a reference gene. Lo states, "The single-copy nature of the sequence chosen was based on Southern blotting and co-amplification experiments, in which both the Y-sequence and α-antitrypsin sequence were amplified to the same extent." One would expect that for a fetus containing an extra Y-chromosome that the Y-sequence described by Lo would be amplified twice (once on the normal Y-chromosome and again on the extra aneuploid Y-chromosome). The detecting of twice as much Y chromosome DNA compared to a normal value would indicate the presence of the extra aneuploid Y chromosome. *Kazakov Decl. at ¶¶ 59 (Ex. 1046 - AT).*

E. Claims 3, 12, 13, 15 and 18 Are Rendered Obvious by *Simpson*, *Schallhammer*, *Kazakov* and *Bianchi*

The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is rendered obvious by *Simpson* (*Ex. 1025 -X*), *Schallhammer* (*Ex. 1022 - V*) and *Kazakov* (*Ex. 1014 - N*). (*Decision, pp. 30-32, and 37, Ex. 1042 - AN*). The claim chart below sets forth the correspondence between the claims of the '540 Patent and the combined teachings of *Simpson (Ex. 1025 -X), Schallhammer (Ex. 1022 - V), Kazakov (Ex. 1014 - N)* and *Bianchi* (*Ex. 1043 - AP*). It should be noted that the *Simpson* reference attached as Appendix 1025 -X is considered cumulative with respect to the claimed subject matter with the 1993 *Simpson* reference attached as Appendix 1024 X-0. As shown below, claims 3, 12, 13, 15 and 18 are rendered obvious by the combination of *Simpson*, *Schallhammer*, *Kazakov* and further in view of *Bianchi*.

US 6,258,540 Claim Language	**Correspondence to *Simpson* (*Ex. 1025 - X*), *Schallhammer* (*Ex. 1022 - V*), *Kazakov (Ex. 1014 - N)*, and *Bianchi* (*Ex. 1043 - AP*)**
Claim 1: A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female,	The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is obvious in light of *Simpson* (*Ex. 1025-X*), *Schallhammer (Ex. 1022 - V), Kazakov* (*Ex. 1014 - N*). (*Decision, pp. 30-32, and 37, Ex. 1042 - AN*). The corresponding findings in the Decision are incorporated herein by reference. The original declarations of Barker, Kazakov, Mansfield, and

which method comprises amplifying a paternally inherited nucleic acid from the serum or plasma sample and detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample.	Vasioukhin, submitted herewith as Exhibits 1005-1008, set forth a *prima facie* case of unpatentability for claim 1 for the same reasons detailed in the 1st petition.
2. The method according to claim 1, wherein the foetal nucleic acid is amplified by the polymerase chain reaction.	*Simpson* teaches amplification of Y chromosome sequences from fetal DNA: "Polymerase chain reaction (PCR) technology has enabled the detection of fetal sex" and "Polymerase chain reaction (PCR) to verify fetal origin" (*Ex. 1025 -X*, respectively, abstract & section bridging cols. 1-2 on p. 1230). *Kazakov* teaches amplifying Alu and inter-Alu sequences present in maternal serum or plasma using the polymerase chain reaction. (*Ex. 1014 - N*, p. 233, "Material and Method"). *Kazakov* inherently amplifies fetal Alu and inter-Alu sequences since such sequences are ubiquitous in both the fetal and maternal genome. *Bianchi* teaches PCR for amplifying fetal DNA in a sample of mixed maternal and fetal DNA obtained from isolated blood cells: > STUDY DESIGN: Samples from 40 pregnant women were flow sorted with different monoclonal antibodies. Deoxyribonucleic acid was subsequently purified from candidate fetal cells; polymerase chain reaction was performed with synthetic primers specific for sequences on chromosomes Y and 7. (*Ex. 1043 - AP*, abstract). It would have been obvious to use PCR to amplify paternally inherited nucleic acid in a maternal plasma or serum sample

	since *Simpson*, *Kazakov* and *Bianchi* each expressly teach using PCR to amplify fetal DNA exclusively of paternal origin such as Y chromosome DNA, or to amplify fetal Alu or inter-Alu sequences about half of which would originate with the father. (*Mansfield Decl. at ¶ 146*)(*Ex. 1047 - AU*).
3. The method according to claim 2, wherein at least one foetal sequence specific oligonucleotide primer is used in the amplification. 5. The method according to claim 1, wherein the presence of a foetal nucleic acid sequence from the Y chromosome is detected.	*Simpson* teaches Y chromosome primers for selective amplification of Y chromosome sequences specific to a male fetus (*Ex. 1025 -X*, p. 1230, section bridging cols. 1-2). *Bianchi* teaches male fetus specific oligonucleotide primers that amplify Y chromosome nucleic acids: "Male fetal cell specific polymerase chain reaction reactions were set up with DNA primers that are complementary to a sequence, on the Y chromosome, Y49a." (*Bianchi*, *Ex. 1043 - AP*, p. 923, col. 2, last para); *see also Kazakov Decl. at ¶¶ 31-32* (*Ex. 1046 - AT*). As explained in the declaration of Dr. Mansfield, it would have been obvious to use the fetal sequence specific primers of *Simpson* or *Bianchi* to amplify fetal DNA exclusively of paternal origin, such as a Y chromosome DNA sequence, for the purpose of identifying the presence of fetal DNA in a maternal plasma or serum (e.g., to confirm pregnancy) or to identify the sex of a male fetus. (*Mansfield Decl. at ¶¶ 146-147*)(*Ex. 1047 - AU*).
12. The method according to claim 5, for determining the sex of the foetus.	See remarks for claim 5; *see also Kazakov Decl. at ¶¶ 36-37* (*Ex. 1046 - AT*). *Simpson* teaches amplification of Y chromosome sequences from fetal DNA: "Polymerase chain reaction (PCR) technology has enabled the detection of fetal sex" (*Ex. 1025 -X*, abstract p. 1229). As explained in the declaration of Dr. Mansfield, it would have been obvious to use the Y chromosome fetal sequence specific primers of *Simpson* or *Bianchi* to amplify fetal DNA of paternal origin in a male fetus for the purpose of identifying the sex of a male fetus. (*Mansfield Decl. at ¶¶ 148*)(*Ex. 1047 - AU*).
13. The method	In the combined method the concentration of fetal nucleic acid sequence from the Y chromosome is measured according to the

according to claim 5, which comprises determining the concentration of the foetal nucleic acid sequence in the maternal serum or plasma.	technique described in *Bianchi*. *See Kazakov Decl. at ¶¶ 47, 48 and 55-57* (*Ex. 1046 - AT*) and *Mansfield Decl. at ¶¶ 135*)(*Ex. 1047 - AU*). *Bianchi* uses quantitative PCR to determine a ratio of (fetal DNA) to (fetal DNA + maternal DNA), which is the relative concentration of fetal DNA in the DNA sample. *Kazakov Decl. at ¶¶ 47, 48, and 55-57* (*Ex. 1046 - AT*). *Bianchi* quantitates and compares the amount of amplified Y chromosome DNA and the amount of amplified Chromosome DNA as follows: > Gel analysis and quantitation. Amplified products were loaded onto 2% Seakem ME agarose gels (FMC Bioproducts, Rockland, Me.) and separated by electrophoresis at a constant current (175 mA for 1.5 hours. The gels were subsequently dried and exposed to a phosphorimager (Molecular Dynamics, Sunnyvale, Calif.). For each product of the amplification reaction the counts per minute were determined. Male specific fetal cell numbers and total cell numbers were calculated by comparing the degree of label incorporated into sample-specific polymerase chain reaction products with Y-specific and D9-specific standard curves generated independently for every analysis. For a permanent record the dried gel was also exposed to x-ray film (Kodak X-omat, Eastman Kodak, Rochester, N.Y.) at -80°C with intensifying screens for 2 hours. (*Ex. 1043 - AP*, p. 924, col. 1, last para)
15. The method according to claim 13, for the detection of a maternal or foetal condition in which the level of foetal DNA in the maternal serum or plasma is higher or	*Kazakov* teaches that cell death is associated with elevated levels of cell-free DNA in maternal plasma or serum. (*Kazakov*, *Ex. 1014 -N*, p. 233, bottom of first full para). This suggests that conditions exhibiting abnormal cellular proliferation, differentiation and cell death would be characterized by abnormal serum or plasma DNA levels. *Kazakov Decl. at ¶¶ 53-54* (*Ex. 1046 - AT*); (*Mansfield Decl. at ¶¶ 116, 119-123*)(*Ex. 1047 - AU*).

lower than normal.	As explained in the declarations of Kazakov and Mansfield, a skilled artisan would have been motivated to determine the concentration of fetal DNA in a maternal serum or plasma sample using the methods of *Bianchi* and compare that to a "normal" value for the purpose of detecting a maternal or fetal condition characterized by a higher or lower level fetal DNA in the serum or plasma. *See Kazakov Decl. at ¶¶ 52-54* (*Ex. 1046 - AT*) and (*Mansfield Decl. at ¶¶ 136*)(*Ex. 1047 - AU*). *Bianchi* describes detection of fetal aneuploidy, which is a condition in which the relative concentration of a particular chromosome would be elevated compared to a non-aneuploid state. (*Ex. 1043 - AP*, Abstract and p. 922, col. 1, first para). As explained in the Declaration of Professor Kazakov, this provides further motivation for determining the concentration of fetal DNA in plasma or serum relative to a normal, non-aneuploid pregnancy at the same stage of gestation. *Kazakov Decl. at ¶¶ 52-54* (*Ex. 1046 - AT*). One of ordinary skill in the art would have been motivated to determine the concentration of fetal DNA in a maternal serum or plasma sample using the quantitative PCR method of *Bianchi* and compare that to a "normal" value for the purpose of detecting a maternal or fetal condition characterized by a higher or lower level fetal DNA in the serum or plasma based. *Mansfield Decl. at ¶ 150*)(*Ex. 1047 - AU*).
18. The method according to claim 13, for detection of a foetal chromosomal aneuploidy.	*Bianchi* discloses a method for detection of fetal aneuploidy: > The existence and persistence of fetal cells in the maternal circulation is no longer considered controversial. The detection of fetal aneuploidy, fetal gene mutations, fetal polymorphisms, and fetal gender by many investigators working independently has demonstrated the feasibility of prenatal genetic diagnosis by maternal venipuncture." (*Bianchi*, *Ex. 1043 - AP*, p. 922, 1st para) *Simpson* similarly teaches detection of fetal aneuploidy: > Fetal aneuploidy. The best current approach to

	detect fetal aneuploidy involves FISH with chromosome-specific DNA probes. The background frequency of euploid cells with three domains is about 1%. Thus, a significantly higher proportion of cells with three domains should indicate presence of fetal trisomic cells in maternal blood. It was this approach that our group used in 1991 to become the first to detect fetal aneuploidy from analysis of maternal blood (Fig. 2). We analyzed blood taken prior to CVS, first detecting trisomy 18 (Price *et al.*, 1991) and then trisomy 21 (Elias et al., 1992).(*Simpson*, *Ex. 1025 - X*, "Fetal Aneuploidy, p. 1234, col. 2). Simpson specifically describes aneuploidy of the Y chromosome "47,XYY" *(Simpson, Ex. 1025, p. 1229, Summary)* As explained in the declaration of Dr. Mansfield, skilled artisans would have considered it obvious to detect a fetal chromosomal aneuploidy by detecting a lower or higher than normal (non-aneuploid) concentration of fetal DNA using the quantitative PCR methods of *Bianchi* to determine the relative concentrations of different chromosomes in a maternal plasma or serum sample. (*Mansfield Decl. at ¶ 150*)(*Ex. 1047 - AU*).

F. Claims 3, 12, 13, 15 and 18 Are Rendered Obvious by *Simpson*, *Schallhammer, Kazakov* and *Mutter*

The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is rendered obvious by *Simpson* (*Ex. 1025 -X*), *Schallhammer* (*Ex. 1022 - V*) and *Kazakov* (*Ex. 1014 - N*). (*Decision, pp. 30-32, and 37, Ex. 1042 - AN*). The claim chart below sets forth the correspondence between the claims of the '540 Patent and the combined teachings of *Simpson (Ex. 1025 -X), Schallhammer (Ex. 1022 - V), Kazakov (Ex. 1014 - N)* and *Mutter* (*Ex. 1048 - AS*). As shown below, claims 3, 12, 13, 15 and 18 are rendered obvious by the combination of *Simpson*, *Schallhammer, Kazakov* and further in view of *Mutter*.

US 6,258,540 Claim Language	**Correspondence to *Simpson* (*Ex. 1025 - X*), *Schallhammer (Ex. 1022 - V)*, *Kazakov (Ex. 1014 - N)*, and *Mutter* (*Ex. 1048 - AS*)**
1. A method for detecting a paternally inherited nucleic acid of fetal origin performed on a maternal serum or plasma sample from a pregnant female, which method comprises amplifying a	The Decision on the 1st Petition found that Petitioner had a reasonable likelihood of prevailing on the ground that claim 1 is obvious in light of *Simpson* (*Ex. 1025-X*), *Schallhammer (Ex. 1022 - V), Kazakov* (*Ex. 1014 - N*). (*Decision, pp. 30-32, and 37, Ex. 1042 - AN*). The corresponding findings in the Decision are incorporated herein by reference. The original declarations of Barker, Kazakov, Mansfield, and Vasioukhin, submitted herewith as Exhibits 1005-1008, set forth a *prima facie* case of unpatentability for claim 1 for the same reasons detailed in the 1st petition.

paternally inherited nucleic acid from the serum or plasma sample and detecting the presence of a paternally inherited nucleic acid of fetal origin in the sample.	
2. The method according to claim 1, wherein the foetal nucleic acid is amplified by the polymerase chain reaction.	*Simpson* teaches amplification of Y chromosome sequences from fetal DNA: "Polymerase chain reaction (PCR) technology has enabled the detection of fetal sex" and "Polymerase chain reaction (PCR) to verify fetal origin" (*Ex. 1025 - X*, respectively, abstract & section bridging cols. 1-2 on p. 1230). *Kazakov* teaches amplifying Alu and inter-Alu sequences present in maternal serum or plasma using the polymerase chain reaction. (*Ex. 1014 - N*, p. 233, "Material and Method"). *Kazakov* inherently amplifies fetal Alu and inter-Alu sequences since such sequences are ubiquitous in both the fetal and maternal genome. *See also Kazakov Decl. at ¶¶ 9-30* (*Ex. 1046 - AT*). *Mutter* teaches amplifying Y chromosome DNA containing the Y chromosome marker ZFY (*Mutter*, *Ex. 1048 - AS*, Abstract, p. 4204, "PCR Amplification"). As explained in the declarations of Kazakov and Mansfield, it would have been obvious to use PCR to amplify paternally inherited nucleic acid in a maternal plasma or serum sample in view of *Kazakov* and *Mutter*. *See Kazakov Decl. at ¶¶ 35, 37, 38, 40 and 45* (*Ex. 1046 - AT*) and (*Mansfield Decl. at ¶¶ 139, 155*)(*Ex. 1047 - AU*).
3. The method according to claim 2, wherein at least one foetal sequence specific oligonucleotide primer is used in the	*Simpson* teaches Y chromosome primers for selective amplification of Y chromosome sequences specific to a male fetus (*Ex. 1025 - X*, abstract, p. 1230, section bridging cols. 1-2). *Mutter* teaches X and Y chromosome specific primers (*Mutter*, *Ex. 1048 - AS*, abstract, p. 4204, "PCR Amplification"); *see*

amplification.	*also Kazakov Decl. at ¶¶ 35, 37, 38, 40, 45* and 46 (*Ex. 1046 - AT*).
5. The method according to claim 1, wherein the presence of a foetal nucleic acid sequence from the Y chromosome is detected.	*Kazakov* uses primers that amplify paternally inherited fetal Alu and inter-Alu sequences in a maternal plasma or serum sample. *(See discussion above in connection with claim 2).* As explained in the declarations of Kazakov and Mansfield, it would have been obvious to substitute the X or Y chromosome primers described by Mutter for the Alu and inter-Alu primers of *Kazakov* because both kinds of primers amplify paternally inherited DNA. *See Kazakov Decl. at ¶¶ 9-30, 35, 37, 38, 40, 45* and 46 (*Ex. 1046 - AT*); (*Mansfield Decl. at ¶¶ 139-140 and 153-154*)(*Ex. 1047 - AU*). One would have had a reasonable expectation of success for amplifying fetal DNA from maternal plasma or serum because *Kazakov* teaches there are only two sources for this DNA—either the mother or the fetus. *See Kazakov Decl. at ¶¶ 35, 37* (*Ex. 1046 - AT*).
12. The method according to claim 5, for determining the sex of the foetus.	See remarks for claim 5; *see also Kazakov Decl. at ¶ 48* (*Ex. 1046 - AT*). As explained in the declarations of Kazakov and Mansfield, it would have been obvious to use the Y chromosome fetal sequence specific primers of *Simpson* or *Mutter* to amplify fetal DNA of paternal origin in a male fetus for the purpose of identifying the sex of a male fetus. *See Kazakov Decl. at ¶¶ 9-30 and 46* (*Ex. 1046 - AT*); (*Mansfield Decl. at ¶¶ 30 and 155*)(*Ex. 1047 - AU*).
13. The method according to claim 5, which comprises determining the concentration of the foetal nucleic acid sequence in the maternal serum or plasma.	In the combined method of *Kazakov* and *Mutter*, the concentration of foetal nucleic acid sequence from the Y chromosome is measured according to the technique described in *Mutter*. *See Kazakov Decl. at ¶¶ 47 and 49* (*Ex. 1046 - AT*); (*Mansfield Decl. at ¶ 142*)(*Ex. 1047 – AU*). *Mutter* teaches a method for determining the relative concentration of nucleic acid sequences from the X chromosome and from the Y chromosome. (*Mutter*, *Ex. 1048 - AS*, abstract, "PCR Amplification").

	Alternatively, if claim 13 is more broadly interpreted to cover determining the concentration of the total fetal DNA in the maternal serum or plasma, it would be obvious to calculate the absolute concentration of fetal DNA in the sample by simply multiplying the relative concentration of fetal DNA (i.e., the percentage of fetal DNA in the sample) by the total concentration of DNA in the DNA sample. (*Mansfield Decl. at ¶¶ 142 and 156*)(*Ex. 1047 – AU*).
15. The method according to claim 13, for the detection of a maternal or foetal condition in which the level of foetal DNA in the maternal serum or plasma is higher or lower than normal.	Simpson specifically describes aneuploidy of the Y chromosome "**47,XYY"** (*Simpson, Ex. 1025, p. 1229, Summary*). *Kazakov* indicates that cell death is associated with elevated levels of cell-free DNA in maternal plasma or serum. (*Kazakov*, Ex. *1014 - N*, p. 233, bottom of first full para). Thus, *Kazakov* suggests that conditions exhibiting abnormal cellular proliferation, differentiation and cell death would be characterized by abnormal serum or plasma DNA levels. *Kazakov Decl. at ¶¶ 52 and 53* (*Ex. 1046 - AT*); (*Mansfield Decl. at ¶¶ 117-121*)(*Ex. 1047 - AU*). Thus, *Kazakov* suggests that conditions exhibiting abnormal cellular proliferation, differentiation and cell death would be characterized by abnormal serum or plasma DNA levels. *Mutter* describes conditions where the level of fetal DNA in the maternal serum or plasma is higher or lower than normal. For example, fetal DNA in maternal serum of a woman carrying an aneuploid foetus, such as those described on page 4203, col. 2, 1st paragraph of *Mutter*, would be higher than for a mother carrying a normal, non-aneuploid fetus due to the presence of extra X or Y chromosomes in aneuploid fetus, e.g. in a foetus having the"47,XXX" or "47,XYY" genotype. One of ordinary skill in the art would have been motivated to determine the concentration of fetal DNA in a maternal serum or plasma sample using the quantitative PCR method of *Mutter* and compare that to a "normal" value for the purpose of detecting a maternal or fetal condition characterized by a higher

	or lower level fetal DNA in the serum or plasma. *See Kazakov Decl. at ¶¶ 51-57* (*Ex. 1046 - AT*) and (*Mansfield Decl. at ¶¶ 143 and 157*)(*Ex. 1047 - AU*).
18. The method according to claim 13, for detection of a foetal chromosomal aneuploidy.	*Simpson* and *Mutter* each describe the detection of aneuploid conditions. Simpson and Mutter specifically describes aneuploidy of the Y chromosome "47,XYY" (*Simpson, Ex. 1025, p. 1229, Summary, Mutter, Ex. 1046, p. 4203, col. 2, para. 1*). *Mutter* describes aneuploid conditions where the level of DNA in an aneuploidy foetus would be higher or lower than normal. For example, the level of fetal DNA in the blood of a woman carrying an aneuploid foetus, such as those described on page 4203, col. 2, 1st paragraph of *Mutter*, would be expected to be higher than for a mother carrying a normal, non-aneuploid fetus due to the presence of extra X or Y chromosomes in aneuploid fetus, e.g. in a foetus having the"47,XXX" or "47,XYY" genotype. > Fetal aneuploidy. The best current approach to detect fetal aneuploidyinvolves FISH with chromosome-specific DNA probes. The background frequency of euploid cells with three domains is about 1%. Thus, a significantly higher proportion of cells with three domains should indicate presence of fetal trisomic cells in maternal blood. It was this approach that our group used in 1991 to become the first to detect fetal aneuploidy from analysis of maternal blood (Fig. 2). We analyzed blood taken prior to CVS, first detecting trisomy 18 (Price *et al.*, 1991) and then trisomy 21 (Elias etal., 1992). (*Simpson*, *Ex. 1025 - X*, "Fetal Aneuploidy, p. 1234, col. 2). It would have been obvious to detect a fetal chromosomal aneuploidy by detecting a lower or higher than normal (non-aneuploid) concentration of fetal DNA using the quantitative PCR methods of *Bianchi* to determine the relative concentrations of different chromosomes in a maternal plasma

	or serum sample. (*Mansfield Decl. at ¶¶ 144 and 158*)(*Ex. 1047 - AU*).

G. Claim 8 is Anticipated by Kazakov

The 1st Petition urged that claim 8 was anticipated since "the Kazakov method 'detected' paternally inherited non-Y chromosomes because Alu repeats on non-Y chromosomes would have been amplified by the B1 and C2 primers used by Professor Kazakov." The Decision on the 1st Petition found that Petitioner "had not set forth a reasonable likelihood that claim 8 is anticipated by Kazakov" because "it is unclear what was amplified by the B1 and C2 primers described in the Kazakov reference." *(Decision at 26)*

The second declaration of Dr. Mansfield demonstrates that the samples from pregnant women similar to those utilized in the Kazakov reference inherently contained fetal extracellular DNA, as sequences from the Y chromosome could be amplified and detected using the polymerase chain reaction (PCR). *(Mansfield Decl. at ¶69)*(*Ex. 1047 - AU*). Thus, extracellular fetal DNA in these samples was available to serve as an amplification template using PCR. *(Id.)*.

The second declaration of Prof. Kazakov submitted herewith demonstrates that the B1 and C2 primers utilized in the Kazakov 1995 reference also would have resulted in an amplification product from non-Y chromosomes when using the

serum of pregnant women as the template. Dr. Kazakov's declaration submits and describes an *in silico* search of a public database for genomic regions complementary to the B1 and C2 primers which demonstrates that the use of the B1 and C2 primers in a PCR amplification of genomic DNA would have resulted in products from multiple non-Y chromosomes. *(Kazakov Decl. at 81-82)* Dr. Kazakov annotated the database searches to identify certain of the non-Y sequences amplified by the B1 and C2 primers. (*Id.*) Because the samples used by Kazakov would have inherently contained fetal nucleic acids, this evidence demonstrates that the amplification procedure using the B1 and C2 primers would have generated products from both paternally-inherited and maternally-inherited fetal DNA as well as from the maternal genome. *(Id.)*

In view of this supplemental evidence, Petitioner respectfully submits that it has now demonstrated a reasonable likelihood that claim 8 is anticipated by Kazakov.

VIII. CONCLUSION

There is a reasonable likelihood that Petitioner will prevail in demonstrating that each of claims 3, 8, 12, 13, 15 and 18 is unpatentable. *Inter Partes* Review of claims 3, 8, 12, 13, 15 and 18 is accordingly requested.

Respectfully submitted,
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Dated: April 19, 2013

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CERTIFICATE OF SERVICE

The undersigned hereby certifies that a copy of this Petition for *Inter Partes* Review and supporting materials at the correspondence addresses of record for the '540 patent was served on the following by overnight delivery:

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